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CD-RAP/MIA - a marker of chondrogenesis in equine bone marrow-derived mesenchymal stem cells

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Purpose: Studies have shown that equine adult mesenchymal stem cells (MSCs) can differentiate into chondrogenic cells for the potential treatment of injured articular cartilage. CD-RAP (cartilage derived retinoic acid sensitive protein) / MIA (melanoma inhibitory activity) is a small protein secreted by chondrocytes. CD-RAP/MIA is therefore available for analysis in the culture medium making it possible to monitor the chondrogenic process without disturbing the cells. A previous study showed that human MSCs differentiated into chondrogenic cells expressed CD-RAP/MIA. The aim of this study was to evaluate CD-RAP/MIA as a marker of chondrogenesis in equine bone marrow-derived MSCs.
Methods and Materials: Equine MSCs were isolated from bone marrow collected within five minutes post-euthanasia. Second passage MSCs were seeded in pellets of 10^6 cells and chondrogenesis was induced by culturing pellets for 32 days in chondrogenic culture medium containing transforming growth factor β~1~ (TGF-β~1~) or a combination of TGF-β~1~ and insulin-like growth factor 1 (IGF-1). Medium was changed every three days and collected for analysis. After 32 days the pellets were collected and processed for histology or RNA isolation.
Results: Histological analysis showed chondrogenic morphology with increased chondrogenesis in cultures supplemented with both TGF-β~1~ and IGF-1. RT-PCR analysis showed mRNA expression of CD-RAP/MIA and Collagen type 2. Western blot analysis showed presence of CD-RAP/MIA in the culture medium starting from day 21.
Conclusions: The results of this study indicate that CD-RAP/MIA has potential as a marker of chondrogenesis in equine bone marrow-derived MSCs and possibly other types of equine stem cells.

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Perturbation b-catenin signaling in the chondrogenic process of mesenchymal stem cells for the generation of phenotypically stable cartilage tissue

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Purpose: Human mesenchymal stem cells (MSCs) have the potential to undergo full span chondrogenic differentiation resembling endochondral ossification. Effective use of MSCs in cell-based therapy for articular cartilage tissue engineering depends hugely on derivation of phenotypically stable hyaline cartilage. This study investigates the effect of temporal perturbation of b-catenin signaling on TGFb-induced MSC chondrogenic differentiation.
Methods and Materials: MSCs derived from human bone marrow were induced to undergo chondrogenic differentiation in a high density pellet system in the presence of TGFb3. Effect of activating or inhibiting the b-catenin pathway was achieved by inclusion of agonist and antagonist. The stages of chondrogenic differentiation were analysed for glycosaminoglycans, type II and type X collagen formation by Alcian blue and immunohistochemical staining and further confirmed by real-time PCR analysis.
Results: Activation of b-catenin signaling elevated the chondrogenic effect of TGFb, resulting in the up-regulated expression of sGAG and type II collagen, and hypertrophic development of the chondrogenic cells. On the other hand, antagonist of b-catenin dose-dependently inhibits the effect of TGFb-initiation of MSC chondrogenesis. When added after the MSC had undergone initial chondrogenesis, b-catenin antagonist exerted the effect of reducing the hypertrophy maturation of chondrocytes.
Conclusions: Our data suggests that TGFb-induced MSC chondrogenesis is dependent on the b-catenin signaling activation. Prolonged activation of b-catenin signaling can, however, lead to hypertrophic differentiation of MSC-derived chondrocytes, which can be inhibited by inclusion of antagonist at the appropriate time during the process of differentiation. By perturbation of the b-catenin pathway, preservation of the chondrocytes at the pre-hypertrophy stage might be achievable.

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The effects of hypoxia on the chondrogenic differenciation of human adipose-derived mesenchymal stem cells

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Purpose: Human adipose tissue-derived stem cells (hATSC) have recently been contemplated as potential reparative cells for cartilage tissue engineering. Chondrogenic differentiation of hATSC can be induced by the combination of enriched culture medium and a three dimensional (3D) environment. Given that cartilage is avascular, oxygen tension has also been suggested as a regulatory factor of chondrocyte differentiation. Our work aimed at determining the effect of hypoxia on the chondrogenic differentiation of hATSC.
Methods and Materials: In this attempt, hATSC were cultured for a 30 day period either in 2D (monolayer) or in 3D (pellets), in control or chondrogenic medium and under low (5%) or normal (20%) oxygen tension. Cell differentiation was monitored at the level of mRNA by real-time PCR. The production of sulfated glycosaminoglycans (GAG) and type II collagen were respectively determined by Alcian blue staining and immunohistological detection on paraffin embedded pellets.
Results: Real-time PCR analysis indicated that type II collagen expression was markedly induced by hypoxia in both culture media. Aggrecan expression was induced by the presence of chondrogenic medium in 2 and 3D culture whatever the oxygen tension. Histological analysis showed the presence of sulfated GAG in chondrogenic medium whatever the oxygen tension. The presence of type II collagen in the matrix of pellets was detected only when hATSC were exposed to chondrogenic medium and hypoxic condition.
Conclusions: Our results highlight the major role of hypoxia and 3D environment in the chondrogenic differentiation of hATSC. Whether our findings may be promising for the cell-based therapy of cartilage should be paid further attention.

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Pharmacological regulation of adult stem cells: Chondrogenesis can be induced using a synthetic inhibitor of the retinoic acid receptor

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Purpose: The use of adult stem cells for the repair of cartilage lesions requires well-defined and efficient protocols for directing the differentiation of stem cells into the chondrogenic lineage. This process currently relies on growth factors that lead to hypertrophy. We have investigated the possibility of using pharmacological manipulation of adult human stem cells by determining which, if any, of the nuclear receptor superfamily might be involved in chondrogenesis.
Methods and Materials: Bone marrow mesenchymal stem cells (BMSCs) from osteoarthritis patients were analysed using RT-PCR and FACS for the expression of nuclear receptor superfamily in monolayer and after chondrogenic differentiation with TGF-β3. Based on this analysis, a synthetic inhibitor of the RAR β receptor (LE135) was used to induce chondrogenic differentiation in 2D and 3D cultures of BMSCs. The chondrogenic tissue was analysed by quantitative RT-PCR and immunoassays. The signalling pathway was analysed by siRNA knockdown of SOX9.
Results: Undifferentiated BMSCs expressed all variants of PPAR, RXR and RARs with little or no RAR γ expression. After chondrogenic stimulation in 3D culture with TGF-β, there was a clear downregulation of RAR β expression. Incubation of BMSCs with LE135 in monolayer or 3D cultures revealed increased aggrecan and type II collagen mRNA and protein content that matched stimulation with TGF-β. SOX9 and type X collagen mRNA was barely detected in LE135 cartilage constructs. SOX9 siRNA knock down inhibited chondrogenesis in TGF-β stimulated monolayer cultures but not in LE135 cultures.
Conclusions: These results demonstrate the feasibility of a pharmacological approach to the regulation of stem cell function.